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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance, a dissertation on, "Some Mercurated Alkyl-Resorcinol Carboxylic Acids" and "The Mercuration of Dinitrofluorescein", submitted by Sidney Charles Overbaugh, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

SOME MERCURATED ALKYL-RESORCINOL CARBOXYLIC ACIDS AND THE MERCURATION OF DINITROFLUORESCEIN

by

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PART I

SOME MERCURATED ALKYL-RESORCINOL CARBOXYLIC ACIDS

Introduction

In recent years there has been a growing interest in organic mercury compounds, as many of them have been found to possess marked bactericidal properties. They have the advantage of not precipitating protein, as do inorganic mercury compounds, and they do not attack metal instruments to any extent.

Special interest has centered around substances containing the hydroxyl group because many of these already possess considerable bactericidal activity, and they lend themselves readily to mercuration. Henry and Sharp[1] were able to prepare mercury derivatives of salicylaldehyde and para-hydroxy-benzaldehyde, although benzaldehyde itself merely reduced the mercury to the mercurous condition.

The influence of alkyl side chains in the benzene ring is well known, since the work of Leonard[2]. It was found that the introduction of longer hydrocarbon chains caused a marked increase in germicidal power until the

hexyl compound was reached. At the same time the toxicity for laboratory animals was considerably diminished.

Henry and Sharp[3] in another investigation mercurated several alkylphenols and phenolic aldehydes. The intensifying effect of the alkyl side-chain is still apparent in the mercury derivatives.

In a later paper[4], the same authors reported on the mercuration of several polyhydroxy-benzaldehydes. Those having two hydroxyl groups in ortho or para position to each other did not yield pure mercury compounds. In the case of 2, 5 - dihydroxybenzaldehyde mercuration was followed by the separation of mercurous acetate owing to intramolecular oxidation and reduction. With 2, 3 - dihydroxybenzaldehyde and 3, 4 - dihydrobenzaldehyde dark-colored mercury compounds were obtained mixed with mercurous acetate or oxide. On dissolving the compounds in sodium hydroxide solution, rapid decomposition took place, with the deposition of metallic mercury.

On the other hand resorcyl-aldehyde or 2, 4 - dihydroxybenzaldehyde having hydroxyl groups in meta position to each other, yielded a stable mercury compound. Its constitution was found to be 3, 5 - diacetoxymercuri - 2, 4 - dihydroxybenzaldehyde by converting it into the known 3, 5 - dibromo-2-hydroxy-4 methoxy-benzaldehyde and 3, 5 - dibromo-4-hydroxy-methoxy-benzaldehyde.

The replacement of the mercuri-group by bromine or iodine according to the method given by Paolini[5] is a method often used to determine its position in the benzene nucleus. This useful reaction can be represented as follows, R - standing for the non-reacting portion of the molecule:-

$$R\text{-}Hg\text{—}OAc + Na\ Br \rightarrow R\text{-}Hg\text{—}Br + Na\ O\ Ac$$

$$R\ Hg\ Br + Br_2 \rightarrow R\ Br + Hg\ Br_2 .$$

Of the compounds prepared several showed pronounced bactericidal action, the most active being the mercury derivative of 2 - hydroxy - 5 - methoxybenzaldehyde. A considerable number of mercurated aromatic acids are known. Benzoic acid can be mercurated, though mercuration does not take place readily[6]. The anhydride of o - hydroxymercuri benzoic acid is obtained, as is shown by the preparation of the same compound by the mercuration of phthalic acid[7]. In the latter case carbon dioxide is evolved and the mercury is joined to the carbon atom left unoccupied.

Introduction of the amino or hydroxyl group into a benzene ring containing a carboxyl group facilitates mercuration. If salicylic acid is boiled with mercuric acetate, 3 - hydroxymercuri -2-hydroxybenzoic acid is

formed[8]. It is believed that in this case mercuric salicylate is first formed which then changes to the above compound. This mechanism of the reaction is supported by the fact that solid mercuric salicylate heated to 100° [9], gives the anhydro - 3 - hydroxymercuri - 2 - hydroxybenzoic acid. The corresponding reaction with mercuric benzoate requires a temperature of 170° C.

A great variety of other hydroxybenzoic acid mercury compounds have been made, as for example, 2 - mercuri - 5 - hydroxybenzoic acid[10] and 2 - mercuri - 4 - hydroxybenzoic acid[11].

Mercurated alkyl resorcinols are also known[12]. Of this series the ethyl and hexylresorcinols were chosen, the latter because of its high phenol coefficient. Like the other compounds mentioned above having hydroxyl groups in meta position to each other they are readily mercurated. Both mono - and di-mercury derivatives were found, which ,though insoluble in ordinary solvents dissolve in sodium hydroxide solution. The effect of the alkyl side chains on germicidal activity in these compounds could not be determined, as unfortunately the alkaline solution decomposed with deposition of metallic mercury.

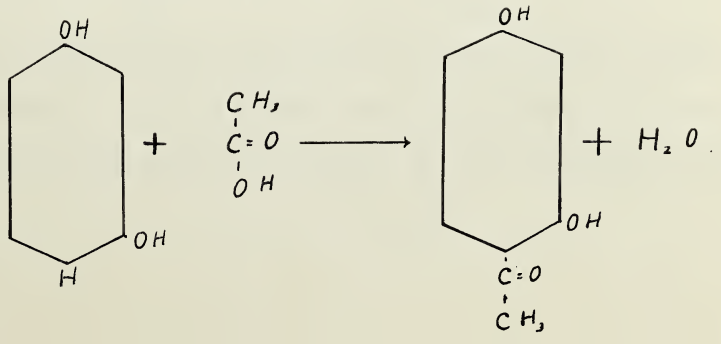
More recently beta - resorcylic acid has been mercurated[13], giving mono - and di - mercury derivatives. The mercury compounds are more stable toward light than the mercurated alkyl-resorcinols. They dissolve more readily in alkali and the solutions show a greater stability.

In view of these facts it was thought that the mercurated alkyl - resorcinol carboxylic acids would perhaps yield mercury compounds which would be stable and owing to the alkyl, side chain and the mercury in the molecule might be effective germicides.

OUTLINE AND DISCUSSION OF INVESTIGATION.

The alkyl resorcinols used in this work were prepared according to the method of Johnson and Hodge[15].

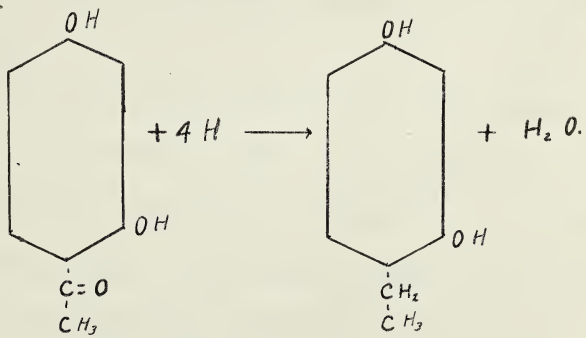
When a fatty acid is heated with resorcinol in the presence of zinc chloride, condensation takes place between the carboxyl group and the hydrogen of the aromatic nucleus. In the case of acetic acid and resorcinol, the reaction is as follows:



The ketone so formed now generally called acetyl resorcinol, was first prepared by Nencki and Sieber[14], and called by them and the older literature resacetophenone , or ethanoylphendiol . The color of the pure ketone (M. P. 142), is not recorded in the literature. As generally prepared it is a dark red solid which may be vacuum distilled to give a yellow oil. The color is probably due to the presence of the compounds resacetein and acetfluorescein formed by further condensation with resorcinol.

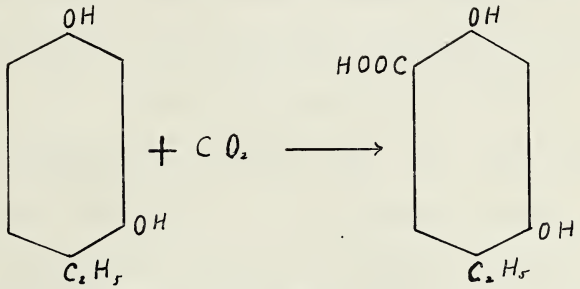
Acetyl-resorcinol is reducible by means of zinc amalgam and hydrochloric acid.

On reduction oxygen is removed from the carboxyl group and the side-chain is reduced to a hydrocarbon one as follows:-



The ethyl resorcinol produced was purified by vacuum distillation. A portion was recrystallized from benzene. Water could not be used as it is too good a solvent.

To introduce the carboxyl group Kolbe's synthesis was used, as in the preparation of beta-resorcylic acid[16]. By this method the compound was dissolved in an aqueous solution of potassium bicarbonate. The solution was heated for some time alone and later with a rapid stream of carbon dioxide passing through.



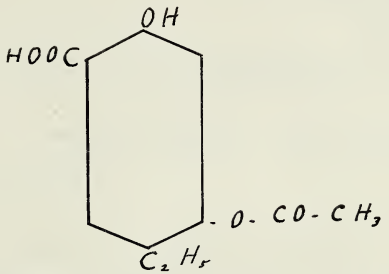
On acidifying the solution while hot, with concentrated hydrochloric acid and allowing it to cool, 2, 4 - dihydroxy - 5 - ethyl benzoic acid crystallized in a thick fibrous mass resembling macerated filter paper in appearance.

This acid is not described in the literature, nor is its preparation given. It is however, mentioned in some biological work by L. Bleyer[17]. It is most readily crystallized from water, being very soluble in hot water and practically insoluble in cold. If the solution is allowed to cool slowly the acid crystallizes in beautiful white needles, which so completely fill the beaker that it may be turned on its side for a few seconds without spilling the mother liquor.

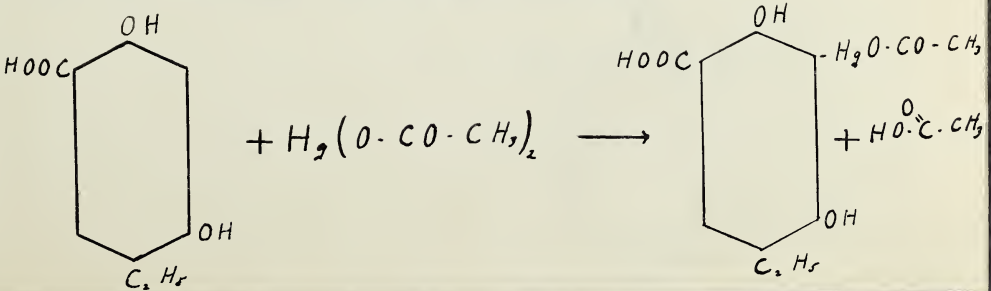
The acid was obtained pure by three recrystallizations. It melts at 166° - 170°C.

The formula proposed is a tentative one, though there is good reason to believe that the carboxyl group is in the position shown.

The monoacetate has been prepared by adding acetic anhydride to the alkaline solution of the acid, according to the method of Lesser and Gad[18]. It has been shown that in this way only the hydroxyl group in para position to the carboxyl group is acetylated. As the hydroxyl group is a much stronger ortho and para directing group than is the ethyl group, the formula of the monoacetate in all probability is:-

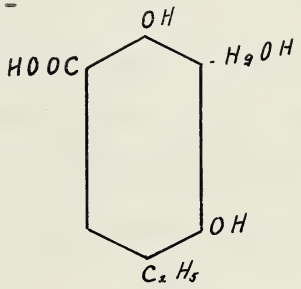


The 2, 4 - dihydroxy - 5 - ethyl - benzoic acid was readily mercurated in dilute alcoholic solution by the theoretical amount of mercuric acetate.



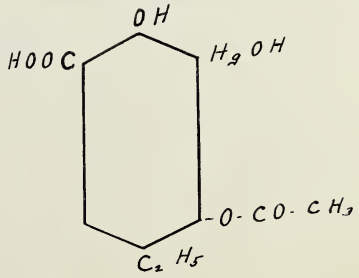
The acetoxy group was immediately hydrolyzed away to give the hydroxymercuri compound which precipitated.

The precipitation took place instantly on the addition of the mercuric acetate, the precipitate which was yellow for a few seconds quickly turned quite white. Analysis showed it to be the 3 - hydroxymercuri- 2, 4 - dihydroxy-5-ethyl benzoic acid, having the following formula:-

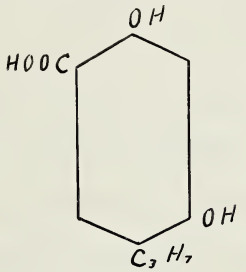


The pure white color of the substance agrees with the hydroxymercuri - formula shown, the anhydro forms often being colored[19].

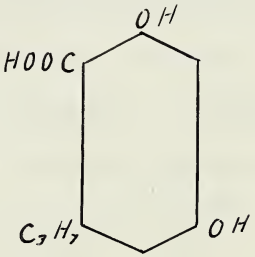
In the same way, by mercurating 5 - ethyl- 2 - hydroxy - 4 acetoxy benzoic acid, the compound 5 - ethyl - 2 - hydroxy- 3 - hydroxymercuri- 4 - acetoxybenzoic acid was obtained having the formula:-



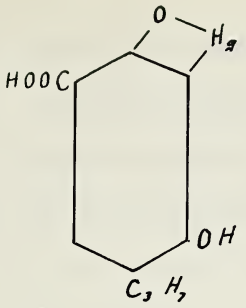
Propionyl resorcinol was prepared similarly to the ethyl compound, using propionic acid instead of acetic. After reduction, the Kolbe synthesis was carried out in a precisely similar manner, to give 2, 4 - dihydroxy - 5 - propyl benzoic acid having the following formula:-



No mention of this acid has been found in the literature, though the closely related divaric acid has been prepared[20]. This substance naturally occuring in lichens is 2 - propyl - 4, 6 - dihydroxy benzoic acid with the following structure:-



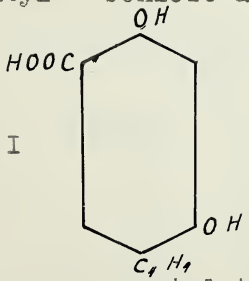
By the mercuration of 2, 4 - dihydroxy - 5 - propyl benzoic acid in dilute alcohol, anhydro - 3 - hydroxymercuri - 2, 4 - dihydroxy - 5 - propyl benzoic acid was obtained as a light yellow solid.



The anhydro structure shown is indicated by the yellow color, as well as by the analysis.

By the above method butyl-resorcinol was prepared by the condensation of n-butyric acid with resorcinol and subsequent reduction.

In this case the carboxylation could not be carried out as in the previous cases, as butyl - resorcinol is insoluble in water. It was carboxylated by the method previously used in the carboxylation of hexyl - resorcinol hereinafter described, to give 2, 4 - dihydroxy - 5 - butyl - benzoic acid, with the following structure;-



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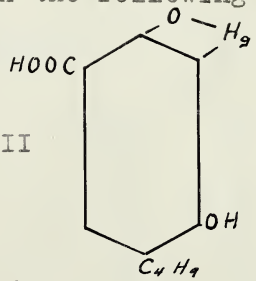
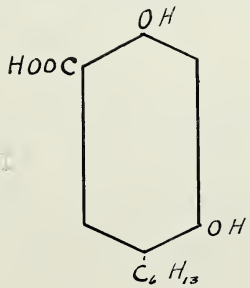


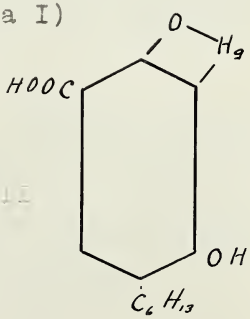
II

This was mercurated to give anhydro - 3 - hydroxymercuri 2, 4 - dihydroxy - 5 butyl - benzoic acid. (Formula II)

Hexyl - resorcinol was prepared as above, using n-caproic acid and resorcinol.

Its carboxylation presented some difficulty owing to its insolubility. Attempts were made to carry out the reaction in aqueous solution, but after eight hours no evidence of reaction was apparent. In an alcohol - water solution sufficient to dissolve a considerable part of the hexyl-resorcinol , negative results were also obtained after several hours refluxing with the passage of carbon dioxide. The method described in Houben - Weyl[21] in the laboratory preparation of salicylic acid was tried. In this, the dry sodium salt of hexyl - resorcinol was heated in an atmosphere of carbon dioxide at atmospheric pressure for several hours without result. The sodium salt in benzene solution also failed to react. The reaction was finally carried out by the method used by Robinson and Robertson in the preparation of p-orsellinic acid[22], using glycerol as the solvent. The acid is tentatively given the formula shown. (Formula I)





It was readily mercurated giving the anhydro- 3 - hydroxymercuri - 2, 4 - dihydroxy - 5 - hexyl benzoic

acid. (Formula II)

It was desired to compare the germicidal power of thesecompounds with their surface tensions in solu - tion. For that purpose solutions of the pure acids were made up by weight, dissolved in the theoretical volume of standard sodium hydroxide or sodium bicarbonate, and made up to fifty or one hundred cubic centimeters.

Surface tensions were measured by two different methods, the capillary rise method, and the direct method by means of the Du Nouy tensiometer. While both methods indicate a steady decrease in surface tension as we ascend the homologous series, the two do not agree. In each case the concentration of the acid used , calculated as the free acid was one part in five hundred parts of solution. The temperature was kept as nearly as possible at 23.0°C.

Phenol coefficients were determined by a modified Rideal-Walker method , using Bacillus typhosus as the test organism.

The results of the surface tension and phenol coefficient measurements are given in the following table. For convenience the acids are referred to under the names of the alkyl radicals present.

TABLE I

Acid used as the Na salt Conc. 1: 500	Surface tension by Capillary Rise dynes/ cm.	Surface Tension by Du Nouy method. dynes/cm.	Phenol coeff.
Water	72.6	73.0	-
Ethyl Acid	72.6	69.0	<0.7
Propyl Acid	72.5	60.8	<0.7
Butyl Acid	70.7	53.6	0.9
Hexyl Acid	65.2	45.5	1.6

As will be observed, the surface tensions measured by the Du Nouy tensiometer are in each case less than those measured by the capillary rise method. As we ascend in the homologous series the surface tensions decrease and the phenol coefficients increase.

It is interesting to compare to phenol coefficients of the alkyl-resorcinol carboxylic acids with those of the uncarboxylated compounds quoted by Dohme, Cox and Miller[23]. This is shown in Table II.

TABLE II

Alkyl-Resorcinols	Phenol Coefficients	Alkyl-Resorcinol Carboxylic Acids	Phenol coeff.
Ethyl-Resorcinol	1.	Ethyl Acid	<0.7
Propyl-Resorcinol	5.0	Propyl Acid	<0.7
Butyl-Resorcinol	22.0	Butyl Acid	0.9
Hexyl-Resorcinol	46.-56	Hexyl Acid	1.6

The phenol coefficient for ethyl resorcinol is not quoted in literature but was obtained by extrapolation on the graph given by the above authors.

It is evident from this that the effect of the alkyl side-chain is still noticeable. The germicidal power however, is greatly reduced by the presence of the carboxyl group.

It is interesting to note that the phenol coefficients reported by various workers , on the same compound, often vary greatly. This variation is due largely to difference in resistance to germicides exhibited by various strains of Bacillus typhosus. It becomes considerable only in the case of a germicide of high potency. For example, hexyl-resorcinol is reported as having coefficients from 52 to 147 , by the same worker using five different strains of test organism.[24]

Nevertheless, it is believed that the comparison made between the alkyl resorcinols and their carboxylic acids is correct, as very little variation occurs with different strains of Bacillus typhosus in phenol coefficients below 5.

It was originally intended to compare the bactericidal potency of the carboxylic acids with that of their mercury derivatives, as in practically every case, the introduction of mercury into a compound intensifies the action. This proved to be impossible however, owing to the instability of the mercury derivatives in solution. Shortly after dissolving in alkali, decomposition took place with the deposition of metallic mercury. As the mercury derivatives are very insoluble in non-reaction solvents, the attempt had to be abandoned. In this respect the compounds described resemble the mercurated alkyl resorcinols.

EXPERIMENTAL PART

Preparation of Acetyl-Resorcinol

A solution containing 90 g. of anhydrous zinc chloride in 120 g. (2.0 moles) of glacial acetic acid was prepared. To this was added 110 g. (1 mole) of resorcinol. The mixture was stirred and kept at the

boiling point for two and one-quarter hours. The mass assumed a deep ruby red color. On pouring this into ten volumes of water , beautiful dark red crystals separated which were allowed to stand a few hours before filtering off. Yield 94 g.

A small portion recrystallized from water yielded red crystals melting at 140 - 141° (uncorr.) On distilling a small portion in vacuo , a light yellow solid was obtained, which was recrystallized from water yielding a product of M.P. 142° (uncorr.)

Pure acetyl-resorcinol is quoted by Nencki and Sieber[25] as having a melting point of 142°.

The remainder of the red crystals was distilled in vacuo at 193 - 200° under 12 mm. pressure. The distillate was a light brown color . It quickly set to a mass of crystals. Yield 71 g.

This was not further purified but was used in the preparation of ethyl resorcinol.

Preparation of Ethyl-Resorcinol

Two hundred and sixteen grams of acetyl-resorcinol was refluxed with 500 g. amalgamated zinc in two litres of hydrochloric acid (Conc. acid and water 1:1). After heating for 12 hours a small portion of the solution no longer gave a red color with ferric chloride, in-

dicating that reduction was complete.

On cooling, a crop of white crystals separated M.P. 95° (uncorr) . These were filtered off, and three more crops obtained by concentrating the solution. As these were contaminated by tarry material, they were distilled in vacuo, boiling at 160° under 12 - 15 mm. pressure. The distillate was nearly white. Total yield 97 g.

Recovery of this compound was difficult owing to its great solubility in water from which it cannot be readily crystallized. It is not volatile with steam. Ether extraction removes zinc chloride as well as ethyl-resorcinol. It can be recrystallized from benzene or toluene.

Carboxylation of Ethyl-Resorcinol

A solution containg 10 g. ethyl-resorcinol and 50 g. potassium bicarbonate in 100 cc. water was heated on the water bath for four hours. It was then refluxed with the rapid passing of carbon dioxide gas for one-half hour. The dark red liquid was acidified while still hot by means of concentrated hydro chloric acid from a dropping-funnel reaching to the bottom of the flask. On cooling, a very bulky fibrous precipitate separated, consisting of fine needles. This was filtered, washed, and recrystallized from water. Yield 4 g.

On repeating this experiment with longer heating ($5\frac{1}{2}$ hours) , and longer passage of carbon dioxide (2 hours), the yield was still 4 g.

On working with larger quantities it was found possible after removal of the first precipitate of the carboxylic acid , to add more potassium bicarbonate, repeat the reaction, and get a second crop of crystals. This was repeated until in all, four crops of crystals had been removed from the same solution.

The acid was recrystallized from water three times, using decolorizing -carbon once or twice as required. The pure acid crystallizes in lustrous needles melting at 166 - 170°. It is very soluble in hot water , though practically insoluble in cold. It is soluble in alcohol and ether.

Analysis, Calcd. : C, 59.3% ; H, 5.54%

Found: C, 59.27% ; H, 5.43%

Mercuration of 2, 4 - Dihydroxy - 5 - Ethyl - Benzoic Acid

A solution containing 7.096 g. (0.039 moles) of the carboxylic acid in 400 cc. water and 65 cc. alcohol was heated on the water-bath. To this was added a hot solution containing 12.44 g. (0.039 moles) of mercuric acetate in 280 cc. water and 20 cc. acetic acid. A

white finely divided precipitate formed immediately. The mixture was heated until the precipitate had become flocculent and begun to settle. It was then filtered, washed with water, and dried at 100°. Yield quantitative.

Analysis, Calcd. : Hg. , 50.30%

Found: Hg., 50.35% ; 50.90%

This compound is a white amorphous solid , insoluble in water and in most ordinary solvents. It is slightly soluble in hot acetic acid , due probably to the formation of the acetoxymercuri compound. It dissolves in sodium hydroxide solution , but the solution is unstable. In a short time a deposit of metallic mercury begins to form, and the solution assumes a brown color. Strong acids quickly remove mercury from the compound as an inorganic salt.

Preparation of the Monoacetate of 2, 4 - Dihydroxy - 5 - Ethyl - Benzoic Acid.

Two grams of the carboxylic acid was dissolved in a few cubic centimeters of approximately normal sodium hydroxide. Acetic anhydride was added dropwise with stirring to the cold solution until excess had been added. After standing a few minutes the cold solution was acidified with hydro chloric acid. The fine crystalline precipitate formed . The mono-acetate was recrystallized twice from 20 % alcohol. Yield , quantitative.

Analysis, Calcd. : C, 58.91% ; H, 5.40%

Found: C, 59.17% ; H, 5.44%

The mono-acetate crystallizes in very fine clusters of needles, easily distinguishable in appearance from its parent compound. M.P. 123 - 143°.

Mercuration of the Mono-Acetate

A hot solution containing 1.20 g. (0.0037 moles) of mercuric acetate was added to a hot solution of 0.85 g. (0.0037 moles) , of the mono-acetate on a water-bath. The solution remained quite clear for several seconds. Then a white finely divided precipitate began to form slowly. The heating was continued as long as the precipitate continued to form (about 30 minutes). The precipitate was filtered off , washed, and dried in the oven. Yield 0.96 g.

Analysis, Calcd.: Hg, 45.41%

Found : Hg, 45.86% ; 46.44%

Like the mercury derivative of the unacetylated acid , this is a white amorphous solid. It is soluble in the same solvents and its alkaline solution is equally unstable.

Preparation of Propionyl-Resorcinol

Two hundred and fifty grams of anhydrous zinc chlor-

ide was dissolved in one hundred and fifty grams (2 moles) propionic acid. (B.P. 135 - 8 uncorr.) To the warm solution was added 100 grams (0.91 moles) resorcinol and the solution was heated to 130° with frequent stirring for 2 hours. On pouring the mass into water , an insoluble red oil was obtained which was washed with water . After standing a few hours it solidified. The propionyl - resorcinol was purified by recrystallization from a large volume of hot water. It crystallized in large orange needles. Yield 63 g.

Preparation of Propyl-Resorcinol

Sixty three grams of recrystallized propionyl - resorcinol was refluxed with 275 g. amalgamated zinc in 700 cc. of dilute hydro chloric acid,(Conc. acid and water 1 : 1). After four hours heating a few drops of the oily layer in 5 cc. alcohol no longer gave a red color with ferric chloride solution. The red oily layer was separated from the aqueous solution . On attempting to wash with an equal volume of water, a clear homogeneous solution resulted. No further attempt was made to isolate the propyl-resorcinol, but the solution obtained was used in the next reaction.

Carboxylation of Propyl-Resorcinol

The solution of propyl-resorcinol obtained as in the last paragraph was mixed with four parts by weight of water and two parts by weight of potassium bicarbonate. This was heated for two hours on a boiling water-bath , and refluxed for two hours with the passage of a rapid stream of carbon dioxide. The solution was diluted and acidified yielding a precipitate of 2, 4 - dihydroxy - 5 - propyl - benzoic acid which was recrystallized three times from hot water. Yield small.

Analysis, Calcd. : C, 61.2% ; H, 6.17%

Found: C, 60.6% ; H, 6.02%

The acid crystallizes in pure white lustrous needles , melting at 177 - 182° (uncorr.) . It is fairly soluble in hot water , practically insoluble in cold. It dissolves readily in alcohol.

Mercuration of 2, 4 - Dihydroxy - 5 - Propyl - Benzoic Acid.

A hot solution containing 2.648 g. (0.0083 moles) of mercuric acetate in 60 cc. water and 1 cc. acetic acid, was added to a hot solution containing 1.630 g. (0.0083 moles) of the carboxylic acid dissolved in 50 cc. water and 12 cc. alcohol. The light yellow precipitate

formed at once. After heating on the water-bath for a few minutes, it was filtered, and the precipitate washed with water and dried at 100°. Yield quantitative (3.2 g.)

Analysis, Calcd. : Hg, 50.81%

Found : Hg, 50.65% ; 49.7%

The yellow color indicates the anhydro structure. In its properties it resembles the corresponding ethyl compound.

Preparation of Butyryl-Resorcinol

One hundred and fifty grams of anhydrous zinc chloride was dissolved in 150 g. (1.7 moles) of n-butyric acid. To the warm solution was added 75 g. (0 .68 moles) resorcinol , and the whole was heated for two hours to 130°. The oil produced was washed in water three times , dried, and vacuum distilled. The product boiled at 190 - 200° under a pressure of 15 to 18 mm. Yield of distilled ketone 77 g.

Preparation of Butyl-Resorcinol

Seventy seven grams of butyryl-resorcinol was refluxed with 800 cc. dilute hydro chloric acid and 300 g. amalgamated zinc. At the end of 23 hours the oil no longer gave a red color with ferric chloride,

indicating complete reduction. The dark red oil was washed three times with water. Yield 67 g. Without further purification the butyl-resorcinol was used in the next preparation.

Carboxylation of Butyl-Resorcinol

Twenty grams butyl-resorcinol in 40 g. glycerol was heated with 40 g. potassium bicarbonate in an atmosphere of carbon dioxide. The temperature was kept near 130°. After heating for three and one half hours, a small test portion dissolved completely in water. The mixture was poured into a litre of cold water and acidified, causing the precipitation of a dark oil, which later solidified. Yield of the crude acid 16 g.

This was recrystallized with 5% alcohol. It crystallizes in white needles and resembles the other acids of the series in appearance. The solid shows a tendency to turn pink, if allowed to stand for some time before filtering. This is probably due to oxidation.

Analysis , Calcd. : C, 62.8% ; H, 6.71%

Found : C, 62.4% ; H, 6.72%

This acid resembles the corresponding propyl compound but is less soluble. It melts 115 -116°. (uncorr.)

Mercuration of 2, 4 - Dihydroxy - 5 - Butyl - Benzoic Acid

A hot solution containing 2.063 g. (0.01 moles) of the carboxylic acid in 80 cc. water and 40. cc. alcohol was prepared. To this was added a solution containing 3.130 g. (0.01 moles) mercuric acetate in 60 cc. water and 1 cc. acetic acid. The precipitate which formed was yellow at first, but on standing turned white. Yield 3.8 g.

Analysis, Calcd. for anhydro compound : Hg, 49.04%

Found: Hg, 48.46% ; 48.36%

In properties this compound resembles the others in the series.

Preparation of Hexylyl-Resorcinol

One hundred and twenty five grams anhydrous zinc chloride was dissolved in 300 g. caproic acid, by warming and stirring. To the solution 95 g. resorcinol was added. The mixture was heated to 125 - 135° for two hours with constant stirring. It was poured into cold water and the oil obtained washed with water, dried over calcium chloride, and distilled in vacuo. The first fraction consisted of caproic acid, boiling at 104 - 5° under 15 to 20 mm. pressure. Hexylyl resorcinol

was collected as a light yellow oil, distilling at 200 - 227° under 12 - 15 mm. pressure. This was again distilled in vacuo, yielding 110 g. of nearly pure ketone. 165 g. of caproic acid was recovered.

Preparation of Hexyl-Resorcinol

One hundred and ten grams hexylyl resorcinol was refluxed with 300 g. amalgamated zinc and 1000 cc. dilute hydrochloric acid. After 13 hours, reduction was complete. The substance was washed with water, dried over calcium chloride and distilled in vacuo. It boiled at 195 - 210° under 12 to 15 mm. pressure. Yield 83.5 g.

Carboxylation of Hexyl-Resorcinol

Fifty grams hexyl-resorcinol was mixed with 100 g. potassium bicarbonate and 100 g. glycerol . The mixture was heated to 130° for three and one-half hours. It was then poured into water and acidified with hydrochloric acid. The oily precipitate solidified. Yield 25 g.

The impure 2, 4 - dihydroxy - 5 - hexyl - benzoic acid was recrystallized from dilute alcohol (20% is suitable) three times . There was considerable loss on recrystallization, and this acid showed a greater tendency to oxidation than the corresponding butyl

compound. This was shown by the rapid assumption of a pink color during crystallization. It crystallized very slowly, two or three days being required. If the filtrate from one crystallization is strongly acidified with hydrochloric acid , the liquid becomes milky in appearance, and a second crop of crystals is formed.

Analysis, Calcd. : C, 65.51% ; H, 7.60%

Found: C, 65.80% ; H, 7.65%

This acid is less lustrous in appearance than the previous one. It crystallizes in short, flexible needles. Like the others, it is soluble in sodium bicarbonate solution . In most solvents it is less soluble than the others of the series. It melts 160 - 164°.

Mercuration of 2, 4 - Dihydroxy - 5 - Hexyl - Benzoic Acid.

The procedure followed was the same as in the case of the butyl compound. Equimolar quantities (1.65 g. of the acid , and 2.209 g. mercuric acetate) were used. The precipitate was light yellow and had the anhydro structure.

Analysis, Calcd. : Hg, 45.93%

Found: Hg, 45.47% ; 45.71%

Surface Tension Measurements

In each case the solutions used in this work were made up accurately by weight. One gram , or in some cases o.5 g. was dissolved in the theoretical amount of standard sodium hydroxide and made up to a 1% solution in a measuring-flask. This was diluted to a concentration of 1 : 500 for use. All concentrations were calculated on the basis of the free acid.

Using the Du Nouy tensiometer , great difficulty was experienced in getting constant readings. When 2 or 3 cubic centimeters on a watch -glass were used, the surface tension as measured decreased steadily, apparently not approaching a constant value.

Much better values were obtained using about 25 cc. of solution in a 50 cc. beaker, the sides of the beaker above the surface helping to shield the liquid from air currents. Using this method, the surface tension as measured was practically constant for solutions whose surface tension did not vary by more than a few dynes per centimeter from that of water.

Out of 27 readings taken on the 2, 4 - dihydroxy - 5 - propyl - benzoic acid in a 1 : 500 dilution, the maximum variation was one dyne each way from the average of 60.8 dynes per cm. The great majority are

much nearer the average.

With solutions of lower surface tension , more variation was observed. When first measured, the surface tension increased rapidly until near the average value, when the variations became smaller, but without any constant tendency in one direction or the other. The surface tension reported for the hexyl compound is the average of 32 readings.

Much more accurate observations were made by the capillary rise method. In this case accurate temperature control was possible, using a litre beaker as a thermostat. The temperature was kept at $23.0^{\circ} \pm .05^{\circ}$ measured by means of a thermometer graduated in tenths of a degree. In every case several measurements were taken of the height of the column of liquid. The meniscus was alternately raised and lowered and allowed to return to equilibrium before each measurement. In most cases the variation in successive readings did not amount to more than 0.01 cm. either way. The maximum variation was in the case of the hexyl compound, the height of the column varying from 7.01 cm. to 7.17 cm.

For purposes of calculation, the capillary rise of pure water was measured. The surface tension at 23.0° obtained from tables is 72.6 dynes per cm. If it is assumed that the density of the solution is the same as that of water which must be very nearly

true for such dilute solutions, the following equation holds : $\gamma_s = \gamma_w \cdot \frac{h_s}{h_w}$ Where "γ" refers to surface tension, "h" to capillary rise , and the subscripts "s" and "w" to salt solution and water respectively. Using this relation the surface tensions of the salt solutions were calculated.

Measurement of Phenol Coefficients

Preparation of Broth

7.5 g. sodium chloride
7.5 g. Lemco Meat Extract
15 g. Peptone Siccum
1500 cc. Distilled water

These ingredients were mixed and autoclaved to insure complete solution. The broth was adjusted to a pH of 7.2 using 1.0 normal sodium hydroxide with the indicator brom thymol blue , and again autoclaved. The precipitated phosphates were filtered off and the broth was put up in 10 cc. portions in 6 x $\frac{5}{8}$ inch broth tubes. After being autoclaved these were ready for use.

Preparation of Phenol Stock-solution

About 60 g. of the purest obtainable of phenol was placed in a dry distilling-flask fitted with a thermometer and a condenser. The first fraction

was discarded. After the constant-boiling portion was reached (B.P. 175 uncorr.) The receiver was replaced by a previously weighed Erlenmeyer flask and approximately 5 g. was collected. The flask was immediately cooled and weighed, the weight of pure phenol being obtained by difference.

The volume of solution required to contain exactly 5.000 g. phenol per 100 cc. was calculated. The phenol was made up to 100 cc. in a measuring-flask. The amount of water required to make up to the desired volume was added from a burette and the whole was thoroughly mixed. This stock solution was used in making up the dilutions required later.

Disinfectant Solution

One gram of the unknown disinfectant was accurately weighed out into a small beaker . The theoretical amount of standard sodium hydroxide solution was added from burette . The solution was rinsed into a measuring-flask and made up to 100 cc. In each case a 1% solution was prepared.

Preparation of Dilutions

I Phenol - For greater accuracy, 30 cc. of each dilution was made up, of which 5 was used. The calculated amount of phenol stock solution was added

to sterile glass-stoppered measuring -cylinders from a burette. Sterile water was added to each to make a total of 30 cc. The dilutions used were as follows:

1 g. phenol in 100 cc. solution
1 g. phenol in 105 cc. solution
1 g. phenol in 110 cc. solution
1 g. phenol in 120 cc. solution
1 g. phenol in 125 cc. solution
1 g. phenol in 130 cc. solution

It was found , after preliminary experiments, which dilution of phenol came within the proper range. Subsequently, only those dilutions in this region were used.

In the experiment, 5 cc. of each phenol solution was transferred to a sterile medication tube by means of a sterile pipette.

II Unknown Disinfectant - A calculated volume of stock solution was transferred to a sterile medication tube by means of a graduated pipette. Using another pipette , the volume of water required to make up 5 cc. of solution was added.

In the first test of an unknown disinfectant , widely separated dilutions were employed, as 1: 100 , 1 : 200, 1 : 500, and 1 : 1000. When the proper range was discovered, dilutions were made up over much narrower limits, as 1 : 150, 1 : 180, 1 : 200, etc.

Dilutions of phenol and disinfectants were made up freshly for use.

Technique

In each run 5 sterile medication tubes each containing 5 cc. of phenol or unknown disinfectant solution were inoculated. The tube containing the most concentrated solution was placed on the left and the concentration steadily decreased toward the right.

In this work, two operators are required, one of whom flames and replugs the tubes after inoculation and the other who inoculates the tubes by means of a pipette or platinum loop .

Starting at zero time the first medication tube on the left was flamed, and inoculated with 0.2 cc. of a twenty-four-hour broth culture of Bacillus typhosus. The tube was gently shaken to insure mixing. Thirty seconds later the operation was repeated on the second medication tube . This was continued until each of the five tubes had been inoculated. Thirty seconds after the addition of the culture to the last tube, a loopful of liquid was withdrawn from the first one, and implanted in a tube of plain broth previously marked "No. 1 ". Thirty seconds later a loopful was withdrawn from the second medication tube and implanted in another broth tube marked "No. 2 ".

This was repeated at precisely the same interval throughout the series of five tubes. After finishing the last one , a second loopful was withdrawn from the first of the series and implanted in broth tube "No. 6 ". This was continued until in all, twenty five broth tubes had been inoculated.

It will be readily seen that from each of the medication tubes loopfuls were withdrawn at intervals of 2.5 minutes, or, counting from time of inoculation with the culture , after 2.5 , 5, 7.5 , 10 , and 12.5 minutes.

Before each operation the platinum loop and the tube to be inoculated were thoroughly sterilized by flaming. After a loopful was withdrawn from the medication tubes, the cotton plug was immediately replaced to minimize the danger of contamination.

The broth tubes were incubated at 37° for 48 hours and examined. Those showing growth were readily detected by the marked cloudiness of the broth. All others remained perfectly clear.

It will be noted that 10 cc. of broth was contained in each tube instead of the 5 cc. recommended by Rideal and Walker. This was to prevent the bacteriostatic action shown by many disinfectants in small concentrations. This results in little or no growth although

the organisms are still living. However, the small amount of disinfectant carried by the loop when mixed with 10 cc. of broth should be so diluted as to have little or no bacteriostatic effect.

As a rule, the phenol in these experiments killed the bacilli in 2.5 minutes in dilutions as high as 1 : 115 , so it was frequently found that the 1 : 120 dilution was the first to show growth. This differs from results previously reported by other workers, using the same strain of Bacillus typhosus, who obtained growth in dilutions as low as 1 : 100 .

In calculating the phenol coefficients the dilution of the unknown disinfectant which killed the organisms in a certain time, was divided by the dilution of phenol acting in the same time.

SUMMARY

1. Carboxylic acid derivatives of some of the alkyl-resorcinols have been prepared.

2. The surface tensions of their sodium salts in aqueous solution have been measured by the capillary-rise method and by the Du Nouy tensiometer.

3. The phenol coefficients of the sodium salts have been measured by a modified Rideal - Walker method.

4. The introduction of the carboxyl group into the alkyl-resorcinol molecule produces a profound lowering of the germicidal activity.

5. Monomercury derivatives of the carboxylic acids have been prepared.

6. Owing to the instability of the mercury derivatives in alkaline solution , no measurement of their germicidal power could be made.

PART II

THE MERCURATION OF DINITROFLUORESCEIN

Introduction

Since the discovery of fluorescein by Baeyer in 1871 , a great number of its derivatives have been prepared. Many substituted fluoresceins are readily made, and these, as well as their parent compound possess many interesting properties.

Owing to the anomalous behaviour of certain of these, a certain amount of confusion has arisen. Von Liebig described five different forms of yellow fluorescein[26]. Orndorff , using specially purified fluorescein, has shown that their are only two forms, a yellow and a red.[27] He was able to prepare only one yellow form, and believes that some of the other varieties reported by Von Liebig , are really impure methoxy derivatives.

The property of existing in two forms is not peculiar to fluorescein alone. Eosin for example, occurs in a colorless form , and in the better known red hydrate.[27] These varieties are explained by Orndorff

on the basis of structure; the colorless eosin, like yellow fluorescein , possessing a lactoid structure, while the more deeply colored forms possess a para-quinoid grouping.

Fluorescein and some of its derivatives form interesting addition compounds with many substances , such as the hydrogen halides , pyridine and alcohol. So far, these have been little studied.

In 1920 White[28] reported the mercuration of a number of phthaleins , including phenol phthalein, fluorescein , phenolsulfonphthalein, o-cresolphthalein and dibromofluorescein. One derivative of the latter hydroxymercuridibromofluorescein, better known as "mercurochrome 220", has come into general use as a disinfectant and urinary antiseptic. This compound has also been shown to be very effective against Bacillus pestis[29].

A mercurated salicylsulfonphthalein has been prepared by Horden[30], and has been shown to kill Bacillus typhosus in dilution of 1 : 2000 in fifteen minutes.

The mercuration of ethyl- and hexyl - fluoresceins has also been reported[31].

The influence of halogen in mercurated sulfon-

phthaleins is shown by the work of Drake and Dunning[32], who tested the bactericidal activity of mono- and di-mercury derivative of dichloro - , dibromo - , and diiodo - resorcinsulfonphthaleins. Resorcinsulfonphthalein itself showed little action. It was not bactericidal in a 1% solution. The introduction of mercury into the molecule caused a marked increase in germicidal effect.

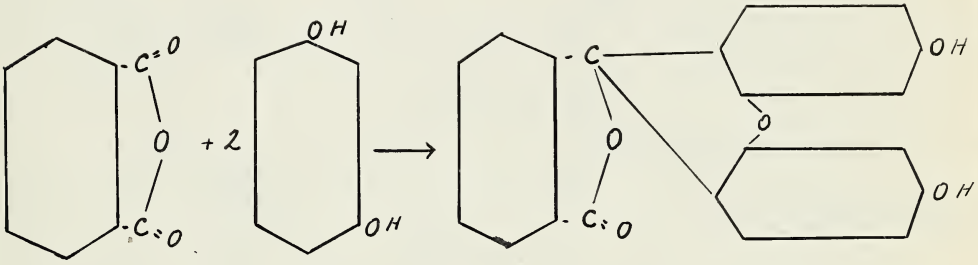
The introduction of halogen gave a product but little more active than the parent substance. However, when both halogen and mercury were introduced the greatest increase in activity was observed. The activating effect of halogen in the mercurated compounds was least in the case of chlorine, and greatest in the case of iodine. The germicidal activity was not proportional to the amount of mercury introduced into the molecule. The introduction of the second mercury atom produced a compound only slightly more efficient than the monomercury derivative. The dimercury compound was also considerably more toxic than the other, to laboratory animals.

Recently, similar monohydroxymercuri - derivatives have been made from di-chloro , di-bromo, and diiodo - fluorescein[33].

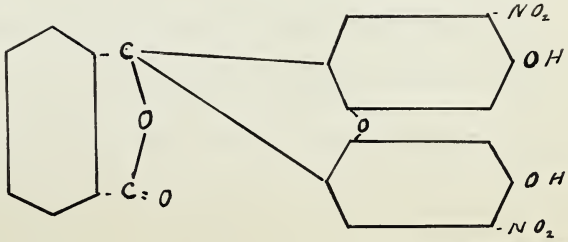
The nitro group is known to have an influence on bactericidal activity in such compounds as p-hydroxy-mercuri-o-nitrophenol. It was thought that possibly the introduction of the nitro group into fluorescein would have an activating effect on the mercury atom subsequently introduced. It was with this possibility in view that the preparation of mercury derivatives of dinitrofluorescein was undertaken.

OUTLINE OF INVESTIGATION

Fluorescein was prepared as recommended by Orndorff[34], using a temperature of 180°. In the presence of zinc chloride phthalic anhydride condenses with resorcinol as shown:

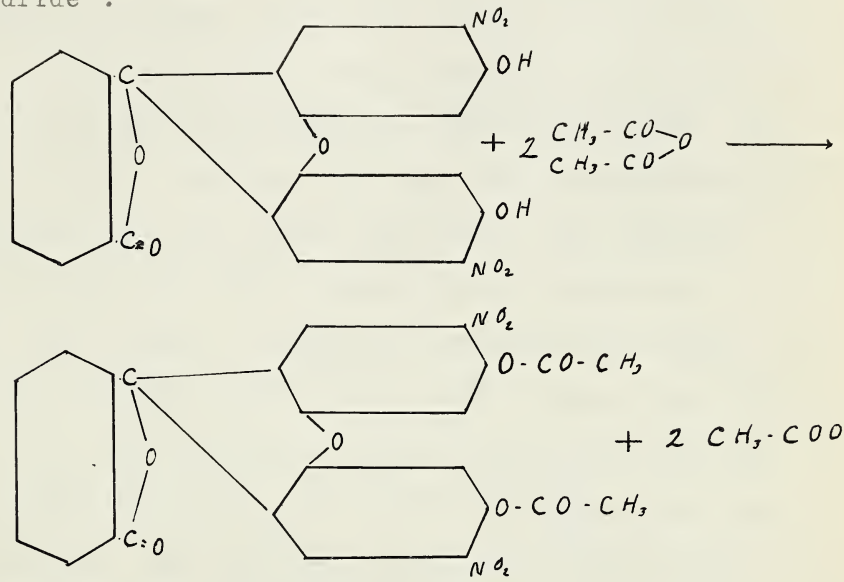


Fluorescein so prepared was readily nitrated in concentrated sulfuric acid solution, by means of fuming nitric acid, to give dinitrofluorescein, possessing the following formula:



Considerable excess of fuming nitric acid must be used. The temperature of the reaction mixture must be kept near zero, or the tetranitro compound results.

The canary yellow flocculent precipitate of impure dinitrofluorescein which was obtained by pouring the reaction mixture into a large volume of cold water, was difficult to purify. This was done by preparing the diacetate by refluxing with acetic anhydride .



Dinitrofluorescein Diacetate

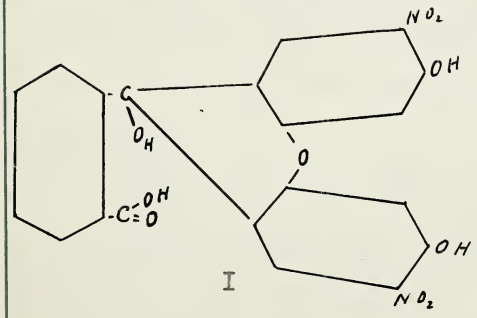
This compound was recrystallized from acetic anhydride in which it is fairly soluble in the hot. After two recrystallizations no further change in

color was noticed, though all was recrystallized three or four times.

Von Baeyer , who first prepared this compound reported it to have a light yellow color, and to be soluble in alcohol. In this investigation no yellow form was obtained , and it appeared to be quite insoluble even in hot alcohol.

By recrystallizing from acetic anhydride two forms were obtained. The first was a light pink crystalline powder. This form always appeared when the solution was fairly concentrated and crystallization started at a moderately high temperature. If a more dilute solution was made , no crystallization commenced until the liquid was cold. The crystals formed were fine white needles which completely filled the liquid. As usually prepared,the second form was slightly pink, though much lighter than the other. On one occasion a small sample was obtained which was paper-white. By controlling concentrations of solutions and the temperature, one form can be readily converted to the other. Both give the same analysis and have the same melting point. For most purposes the pink form is preferable, as it is more easily dissolved.

The purified diacetate was hydrolyzed to yield pure dinitrofluorescein. This was brought about in two ways. By warming with 80% sulfuric acid[36], a clear amber solution was obtained, which, on pouring into cold water yielded a canary yellow precipitate of dinitrofluorescein having the formula shown above. This pure compound was readily obtainable in crystalline form by re-crystallization from alcohol or acetic acid, or simply by allowing it to stand under water for several hours during washing. On hydrolyzing with alkali , a deep reddish violet solution was obtained, which, when acidified yielded a orange yellow precipitate of dinitrofluorescein hydrate . The constitution of this compound has been the basis of some controversy, as two structures are possible.



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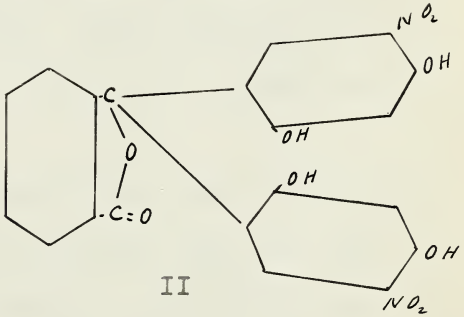


II

The first was accepted soon after the compound was discovered, and is found in the older literature.

Hewitt and Perkins[36] believe that Formula II is correct, as a tetrasodium salt is known. If Formula I were correct, no more than three sodium atoms could be readily introduced.

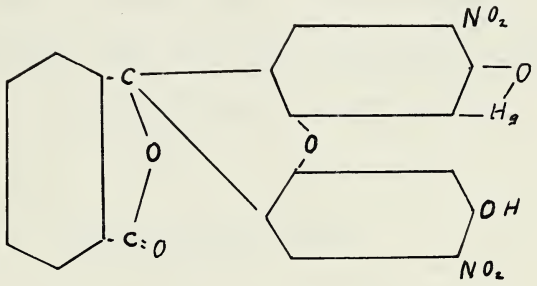
The mercuration of dinitrofluorescein presented some difficulty . When an alcoholic solution of the unhydrated compound was mixed with an equimolar quantity of mercuric acetate solution , a brick red precipitate formed slowly. This invariably contained too much mercury.On analysis it was found to contain about thirty seven per cent mercury, while the theoretical value for the hydroxy mercuri compound is 31.41% . This preparation was carried out five times in all, always with similar results.

An attempt to mercurate dinitrofluorescein in acetic acid was unsuccessful. After heating for several hours, the unchanged dinitrofluorescein was recovered.

It was thought that possibly the dihydrozymercuri derivative formed with such readiness that the precipitate was contaminated thereby. An attempt was made to prepare the dimercury derivative . On adding double the molecular proportion of mercuric acetate

a precipitate was obtained containing only 40% mercury instead of the theoretical 46.8% .

The pure monomercury compound was finally prepared from the impure red precipitate mentioned. On adding a large volume of glacial acetic acid and warming, the precipitate dissolved. The solution was filtered, and refluxed for about an hour. On diluting the solution an orange precipitate formed . This was heated for some time before filtering. It was filtered, washed, and dried at 100°. Analysis showed this to be anhydrohydroxymercuridinitrofluorescein. It dries as an orange red powder having the formula :



BACTERIOLOGICAL WORK

For the purpose of comparison, tests were made on dinitrofluorescein and its monomercury derivative. The former showed no evidence of disinfectant power within the range of the experiment. That is, in a concentration of 1 : 50 ,it failed to kill

Bacillus typhosus in 12.5 minutes. The monomercury derivative in a concentration of 1 : 100 killed the organisms in 5 minutes. In a concentration of 1 : 500 it killed them in 12.5 minutes.

The behaviour of this mercury compound differs considerably from that of the carboxylic acids mentioned in Part I, in that it shows activity over a much greater range of concentration. For example, the carboxylic acid killing the bacilli in 2.5 minutes in a concentration of 1 : 150 , fails to kill them in 12.5 minutes in a concentration of 1 : 200 . In expressing the disinfectant power of compounds which, like the mercurated dinitrofluorescein, act over a wide range , it is usual to give the concentrations and the time required to act, rather than to calculate phenol coefficients.

EXPERIMENTAL PART

Preparation of Fluorescein[34]

One hundred and fifty grams (1 mole) phthalic anhydride was mixed with 220 g. (2 moles) resorcinol, and the mixture was fused on an oil-bath. Seventy grams powdered anhydrous zinc chloride was stirred into the molten mass. The mixture was heated to 180° for two

and one-half hours. At the end of that time the substance was solid. It was cooled, pulverized and extracted with sodium hydroxide solution. After filtering, the solution was acidified with dilute hydrochloric acid , which precipitated the fluorescein. The best results were obtained by precipitating from a warm solution and heating to boiling before filtering. The yellow fluorescein was thereby changed to the less bulky red form which was readily filtered off. Yield 285 g.

Preparation of Dinitrofluorescein[35]

Fifty grams fluorescein was dissolved in 1000 g. concentrated sulfuric acid by gently warming. The solution was cooled to 0 ^{o} and 100 g. (excess) fuming nitric acid was added slowly with vigorous stirring. The addition of the acid was so gradual that the temperature of the mixture never rose above 8^{o}. A gradual lightening of the color was observed as nitration took place. The cold mixture was poured into a large volume of ice water, causing the precipitation of the crude dinitrofluorescein. In this form it was very difficult to wash free of acid. The precipitate was dissolved in a slight excess of alkali and reprecipitated with dilute hydrochloric

acid. The mixture was heated to boiling to coagulate the precipitate before filtering and washing. Yield 50 g.

Preparation of Dinitrofluorescein Diacetate

Ninety eight grams dinitrofluorescein were refluxed with 450 g. acetic anhydride on an oil-bath for four hours. The cooled mixture deposited dark pink crystals of dinitrofluorescein diacetate. After standing for some time in the ice-box the precipitate was filtered off. By evaporating part of the acetic anhydride from the filtrate , and adding alcohol, a second crop of crystals was obtained. Total yield 81 g. On attempting to extract more of the substance from the filtrate, an uncrystallizable syrup was obtained.

The impure dinitrofluorescein diacetate was recrystallized three or four times in small portions, from acetic anhydride. As noted above, a pink and a white form were obtained.

Analysis, Calcd. : C, 56.90% ; H, 2.78%

Found : (pink form) - C, 56.75% ; H, 2.85%

(white form) - C, 56.52% ; H, 2.74%

Both melt at 176 - 8° (uncorr).

Alkaline Hydrolysis of Dinitrofluorescein Diacetate

Five grams of the pure dinitrofluorescein diacetate was heated with 195 cc. of 4% potassium hydroxide solution on a water-bath for one hour. The reddish violet solution was refiltered and acidified with dilute sulfuric acid. The flocculent precipitate was washed by decantation and dried at 100°.

Analysis, Calcd. for hydrate : C, 54.5% ; H, 2.75

Found : C, 54.8% ; H, 2.81% .

Acid Hydrolysis of Dinitrofluorescein

Ten grams dinitrofluorescein diacetate was added to 50 g. of warm 80% sulfuric acid. The mixture was heated on the water-bath for about fifteen minutes before complete solution took place. The canary yellow dye was precipitated by pouring into cold water. This was washed by decantation during which process it gradually became finely divided and crystalline.

Analysis, Calcd. : C, 56.85% ; H, 2.39%

Found : C, 56.99% ; H, 2.46%

Mercuration of Dinitrofluorescein

A solution containing 7.638 g. (0.018 moles) of dinitrofluorescein in 100 cc. alcohol and 50 cc. water was prepared and heated on the water-bath. A hot

solution containing 5.661 g. (0.018 moles) of mercuric acetate in 100 cc. water and 2 cc. acetic acid was added slowly with vigorous stirring. In a few seconds a finely divided brick red precipitate began to form. Heating was continued for one hour and the mixture was allowed to cool. A small quantity of fine white needles was noticed in the upper part of the precipitate. The precipitate was filtered off and dried in the air. Yield 11.3 g.

This preparation was repeated several times. When a larger concentration of acetic acid was present , the precipitate formed much more slowly. As prepared it always contained about 37% mercury.

The crude mercury compound was dissolved in glacial acetic acid and refluxed for one hour. On adding a large volume of water an orange precipitate came down. This was heated for several minutes before filtering. It was filtered and washed with hot alcohol to remove any dinitrofluorescein.

Analysis, Calcd.for Anhydro Compound : Hg, 32.31%

Found : Hg, 31.90% ; 31.86%

Bacteriological Work

The efficiency of the anhydrohydroxymercuri-dinitrofluorescein against Bacillus typhosus was measured, using the method previously described. The stock solution used was made by dissolving 0.5 g. of the substance in the theoretical amount of standard sodium hydroxide, and diluting to 50 cc. in a volumetric flask .

SUMMARY

1. Pure dinitrofluorescein has been prepared.
2. Its monomercury derivative has been made.
3. The bactericidal action of the mercury compound has been measured.

In conclusion, the author wishes to express his sincere thanks to Dr. R.B. Sandin for his assistance and advice, and to Dr. R.M. Shaw , of the Department of Bacteriology, for putting at his disposal the necessary equipment for the bacteriological tests.

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